

Mouse MMAALGPPEVIAQLENAAKVLMAPPMSVMSNE--QRQHAEHIFLFRKSKSPFAVQRHILETSKVDYVLFQAATAIMEAVVREWVLEKGSIEISLRTFLLTIV--LQRPN-L 107
 Zebrafish -MMAAVGAPEVISOLESAAKVLMAAPPMSVMSVTE--QRQHAEHIFLFRKSKSPFAVQKHILETSKVDYVLFQAATAIMEAVVREWVLEKNSIESLRTFLLTIV--LQRPN-L 106
 King cobra -----MNVNNE--QRQHAEHIFLFRKSKSPFAVQKHILETSKVDYVLFQAATAIMEAVVREWVLEKNSIESLRTFLLTIV--LQRPN-L 80
 Maize MQGFFGGADPPQQLQATMLAIEQACSLIQLHM--NPSEAERKVTSLHSSLMFYQVCRFILETSKQPNARFQAAGAIGDAAVREWGVITDDNKRSLIIYICLVN--MEHASSP 108
 D. discoideum -----MEIEFIQNLKFCIGLQSNKSNRETSQSIILTLTKTPQRYKLFNLSKSNKPTIAHFYGLLMIKRSASIREWAAIDSGTKMIIETLFOKIEMNSMNF 100

HEAT 1A HEAT 1B HEAT 2A HEAT 2B HEAT 3A

Mouse QKYVREQILLAVAVIVKRGSLDKSID-----CKSIFHEVSQLISS-GNPTVQTLACSLITALLSEFSSSSKTSNIGLSMFEFGNCKRVFQEDDRQIFMPLTVGLVQEF 209
 Zebrafish -QKYVREQILLAVAVIVKRGSLDKSIN-----CKSIFHEVSQLISS-GNPTVQTLACSLITALLSEFSSSSKTSNIGLSMFEFGNCKRVFQEDDRQIFMPLTVGLVQEF 208
 King cobra -QKYVREQILLAVAVIVKRGSLDKSIE-----CKSIFHEVSQLISS-GNPTVQTLACSLITALLSEFSSSSKTSNIGLSMFEFGNCKRVFQEDDRQIFMPLTVGLVQEF 182
 Maize DGYVQSKVSAVARLLKRGWVEFSDQE-----KAAIFFEVEBSIRGIGHENRQFAAINFLETLVSEFSPST-ASAMSLPKFHFQECYSLEVQFLKDFYCWAQAAV--F 209
 D. discoideum NYATKQGSFNTLVGVIIRKRWLDNEKYEIGKQGMELNQIVMDRVYQYLDLGS-SDRLEIETIKLIGSIIIEFSSSSKAAHIIQLSWEFHOKCLITFONLHQPIFRKVLLELQOF 211

HEAT 3B HEAT 4A HEAT 4B HEAT 5A

Mouse SRREN-LSAQMS-SVFQR---YLALANQVLSWNE-----LPPKLRGRHYIAMEFSSQVLLKFTESWREALLDS---R--VMELFFTVHRK---IREDS 289
 Zebrafish -SRREN-LNAQMS-CVFQR---YLALANQVLSWNE-----LPPNLRGRHYIAMEEATPNVMLKFTESWRESLDH---R--VMDLFFTVHRK---IREDS 288
 King cobra -SRREN-LNAQMS-SVFQR---YLALANQVLSWNE-----LPPKLRGRHYIAMEFSSQVLLKFTESWREALLDS---R--VMELFFTVHRK---IREDT 234
 Maize NTADKILNSVT-IPDERACSAALRLMPQILSWNFKHTVEHESDAKINFLRIDTINLKKFERS---LVKPGSMWRREILISS---GHPTWVNLNFTYLRKYSYDTLVQ 314
 D. discoideum KDHIQQMPSRLTQDSELIOLLYSVKVFDTILDWRF-----LESGSSV-LAYITSESGGRT-NLKPTEIETWISLFTPSQSGGGISFVSLVFLGYLQK---VEKVE 304

HEAT 5B HEAT 6A

Mouse DMAQDSLQCLAAOASLHCPFPDEG---SQVDYLAHFIEGLLNTNGIEI--EDSEAVGISSIISNLIIVFPFRNLTALPSELFSSFNCLTHL---TCSFGRSAALEEVID 393
 Zebrafish -DMAQDSLQCLAAOASLHCPFPDES---AQVYLAHFIEGLLNTNGIEI--EDSEAVGISSIISNLIIVFPFRNLTALPSELFSSFNCLTHL---TCSFGRSAALEEVID 392
 King cobra -DMAQDSLQCLAAOASLHCPVFPDES---SQVDYLAHFIEGLLNTNGIEI--EDSEAVGISSIISNLIIVFPFRNLTALPSELFSSFNCLTHL---TCSFGRSAALEEVID 338
 Maize PIAVSCROLIVQCLSLAGSVFPNDGDAQIKHMLLISAVVLEPPDVTASIRNGGSESEFIDGCHAL-LSAISLTGSLFDNLKSTRPYGTVNLSALTSBAVSKVLN 425
 D. discoideum KIPNLLRHAMSQCGLQCPITIKDK--IKNOYLSVLTFTNKLLEKSIITRNWNEMEDISNIIYKFCNTYKFGIACLPNQIVIPFLQYTFQF---VLS--SLNLKMIWA 407

HEAT 6B HEAT 7A HEAT 7B HEAT 8A

Mouse K---DDM---VYMEAYDKLLESWLTIVRDD-----KHFHKGFFTQHAV---QVFNYSIQCHLAAPDGRNLTANGVASR-----EEEISELQEDDRDQFS 475
 Zebrafish -K---DDM---VYMEAYDKLLESWLTIVQED-----EHFPRGCFVQPAV---QVFNYSIQCHLAAPDGRNLTANGVASR-----EEEISELQEDDRDQFS 474
 King cobra -K---DDM---VYMEAYDKLLESWLTIVQDD-----KHFHKGFFTQHAV---QVFNYSIQCHLAAPDGRNLTANGVASR-----EEEISELQEDDRDQFS 420
 Maize NQSEEBT---WGIDSLDILLETWVNLGQDV-----DADKNPISDGCALASSLPMIVESHKAA---ADSAFEDT---DDTYFHVSVSKRD---504
 D. discoideum KHGEEEBEEEFNDCEPILRSEFVSLISDAEMLNRRKVDLENFKEQYVQLQCS-TSIOYQNYQISRLLESEIEINKSNEELEPTCKSRGGIGGAEDIE---DEDKKKYD 514

HEAT 8B HEAT 9A HEAT 9A

Mouse DOLASVGMGLGRIAAEHCPMLLTSLEERVTRIHGQLRHQQQFLASPGSSITDNKMLDLYDIHHLILVTVGYLLADDTQGETPLIPEIMEYSIKHSSEVDINTTLQILGS 587
 Zebrafish -DOLASVGMGLGRIAADHCIPLLTGLLEDRVTRIHGQLRHQQHLMMAADPDTVRKVLDDLYDIHHLILVTVGYLLADVPQGETPLIPEIMEYSIKHSSEVDINTTLQILGS 586
 King cobra -DOLASVGMGLGRIAAEHCPMLLTSLEEDRVTRIHGQLRH--QQLISSAGSSSIDNKMMLDLYDIHHLILVTVGYLLADDTQGETPLIPEIMEYSIKHSSEVDINTTLQILGS 531
 Maize EOLALYLIARASANTPIFPLAQLFSERFARIN---QRN-----GESDPTQLELYLWLVSHVLTDSGETELLIEALQX-GFPNVIEAA-----589
 D. discoideum EOLRSVAYIGRLNPGQSELELKNENRINRINSK---ERV-----SDPI-----LFLSHLWLVIFAGHLIFDAENKTPSAIENATEDYTFEQCKLTP-----597

HEAT 9B HEAT 10A HEAT 10B HEAT 10A

Mouse PGEKASSIPGYSRTDSVIRLLSAVLRTS-EVESRAIRADLTHLLSPQMGKDIVWFLRRWAKTYLLVDEKLYDQI-----SLPLSTAFGADTEGSQWIIIGYLLQ 684
 Zebrafish -PGEKATSIIPGNCRTDSVIRLLSAVLRTS-EVESRAIRADLTHLLSPQMGKDIVWFLRRWAKTYLLVDEKLYDQI-----SMPLSTAFGADTEGAQWIVGYLLE 683
 King cobra -PGEKASSIPGYSRTDSVIRLLSAVLRTS-EVESRAIRADLTHLLSPQMGKDIVWFLRRWAKTYLLVDEKLYDQI-----SLPLSTAFGADTEGAQWIVGYLLE 628
 Maize -----HHPVVTLSWISINFRSROCLDPCGIRA---KYFSPRLMEAVLFLARWAKTYLLVDEKLYDQI-----SRKLLNSFAWENNOGALVDFVVL 685
 D. discoideum -----ASQVQDGLIDLCNAVFRFMHEYENPLLNNGKMDTISPLVSGTSLWFTSGMSLVLYLPPSSVFNQI-----SPKILEAYGTEQPLLS-ITDYFIN 684

HEAT 11A HEAT 11B HEAT 12A

Mouse KVISNLSVWSSEQDLANDTVQ--LLVTLVERRERANLVICENWNWLAQOFASRSPLNLFSSPVORTLMKALVLGGFAHMDTETTKQOYWTEVQLPQQRFLRVINQENFQOM 795
 Zebrafish -KVINLSVWSSEPELANDTVQ--LLVTLVVEKREANLVICENWNWLAQOFASRSPLHMLSSVORTLMKALVLGGFAHMDSDTKQOYWAEVHLPLQQRFLNLIQENFAQI 794
 King cobra -KVLNSLAVWSSEQELANDTVQ--LLVTLVERRERANLVICENWNWLAQOFARARNPLFLSSSVORTLMKALVLGGFAHMDGDTKQOYWAEVHLPLQQRFLRVINQENFQOI 739
 Maize ISMLALPTYQGENELQTLTCKKLLATVVRKHTCTYLVQLDSWRDLTRAFASGRSLLS-LSGRLQRSIAETLASAASCICKDPEASAQYLRDLMGVPAGCLVENASRSDLSKV 796
 D. discoideum KILLNLKWCSGDLVLAQSN-LNSFTLNKELCKYLIRSPNRSF--FLEG---ISLPPSVYQGLFKAFSRVVEFSF-PLSTRREYFIQLVKTLEVMQDGVGLGRADPTKI 789

HEAT 12B HEAT 13A HEAT 13B HEAT 14A HEAT 14A

Mouse COQEVKQEIATATLEACCGIAEATQI---DNVALF---NFLMDFLNCITGLMEVYKNTPTVNLIIIEVFEVAHKICYLIGESKAMHLYEACLTLQVYSKNNLGRQRID 900
 Zebrafish -COQEVAVKQEIATATLEACCGIAEATQI---DNVASLF---NFLMDFLSSICGLMEVYKNTPTVNLIIIEVFEVAHKICYLIGETKSMKLYEACLTLQVYSKNNLGRKRLD 909
 King cobra -COQEVKQEIATATLEACCGIAEATQI---DNVALF---NFLMDFLNCITGLMEVYKNTPTVNLIIIEVFEVAHKICYLIGESKAMHLYEACLTLQVYSKNNLGRQRID 844
 Maize AQQADVITYMVCCLERLARGAARATQ--RTQKVFLE---EMAHTVMNPLLTLEVYKNSHTVVMYMLKPVVDFVDGQAVFLDAKETSALVSCLOLLQIYSSHNIGKVMLS 901
 D. discoideum SGEAKIKENYIILEKIKNGVSVSESEYVDDDEDDCLFTVDFLTKYATSLIAMIPLYDHCNDIILVLRFSNFTKHLLEYLNQDRARSIFPLIIQLFNSVATTSSHKTKLD 901

HEAT 14B HEAT 15A HEAT 15B HEAT 16A

Mouse VT-----AEEEQVDLLIMELLTNLLSKEFIDF--SDTDEVFRGHEPQGAAGRSVAADVVLYGVNLIPLMS--QDLLKFPFLCNQOYKLIFFICEIFPEKIPQLPEDLFK 1004
 Zebrafish -VA-----AEEQVDLLIMELLTNLLSKEFIDF--SDTDEVLRGQEQSSGARGVSAADVVLYGVNLIPLMS--QDLLKFPFLCNQOYKLIFFICEIFPEKIPQLPEELFK 1003
 King cobra -VT-----AEEQVDLLIMELLTNLLSKEFIDF--SDTDEVFRGHEPQCATNRSVAADVVLYGVNLIPLMS--QDLLKFPFLCNQOYKLIFFICEIFPEKIPQLPEELFK 916
 Maize LSSSLRNSQAERKDLRALLRLTNICSKDLVGLSD-----CGEGSPDIAEVYIIGLDIVTPLIS--LDDLKYPKLSRDYFVLISHLLELYPEKVAHLNSDAFT 1001
 D. discoideum SK-----EYVHRVMMVKLITNIIIT--EGL-----QRNCPPTISETTFPHAINIITCLLENDDLLIYKPLARNYFMITSFPGADNIQVKNIP--VIN 985

HEAT 16B HEAT 17A HEAT 17B HEAT 17B

Mouse SLMYSLELGMTSMSSSEVQCLEALTPA-----EQCAKAQETSDPLFLA-TRHFLKLVFDMVLQKHN--TEMTTAAAGEAFYTLVCLHQAE--SELVETLSS 1099
 Zebrafish -SLMCSLELGMTSMSSSEISQCLEALSPLA-----EQCAKTQEKDTPFLFA-TRHFLKLVFDMVLQKHN--TEMTTAAAGEALYTLVCLHQAE--SELVETLSS 1098
 King cobra -----MVFDMVLQKHN--TEMTTAAAGEAFYTLVCLHQAE--SELVETLSS 960
 Maize RIGSLDFGLRNQDSVVVERCLAAVNALASYNFKERLGGGRRLNSQLMESGSGNGLQESISSHLLDQLLDFEDFR--SELAGYADALDLPLCEQELQORVLEHLLDK 1112
 D. discoideum TIYSLIEAGLHHDLEIVKSCFCIGCLTK-----SLENSKEKSGGLVDPHYQSVLQIGSWINFLLDQFNVDELSSVETLSSVETLSSVSDGGRVIEILITR 1086

HEAT 18A HEAT 18B HEAT 19A HEAT 19B HEAT 20A

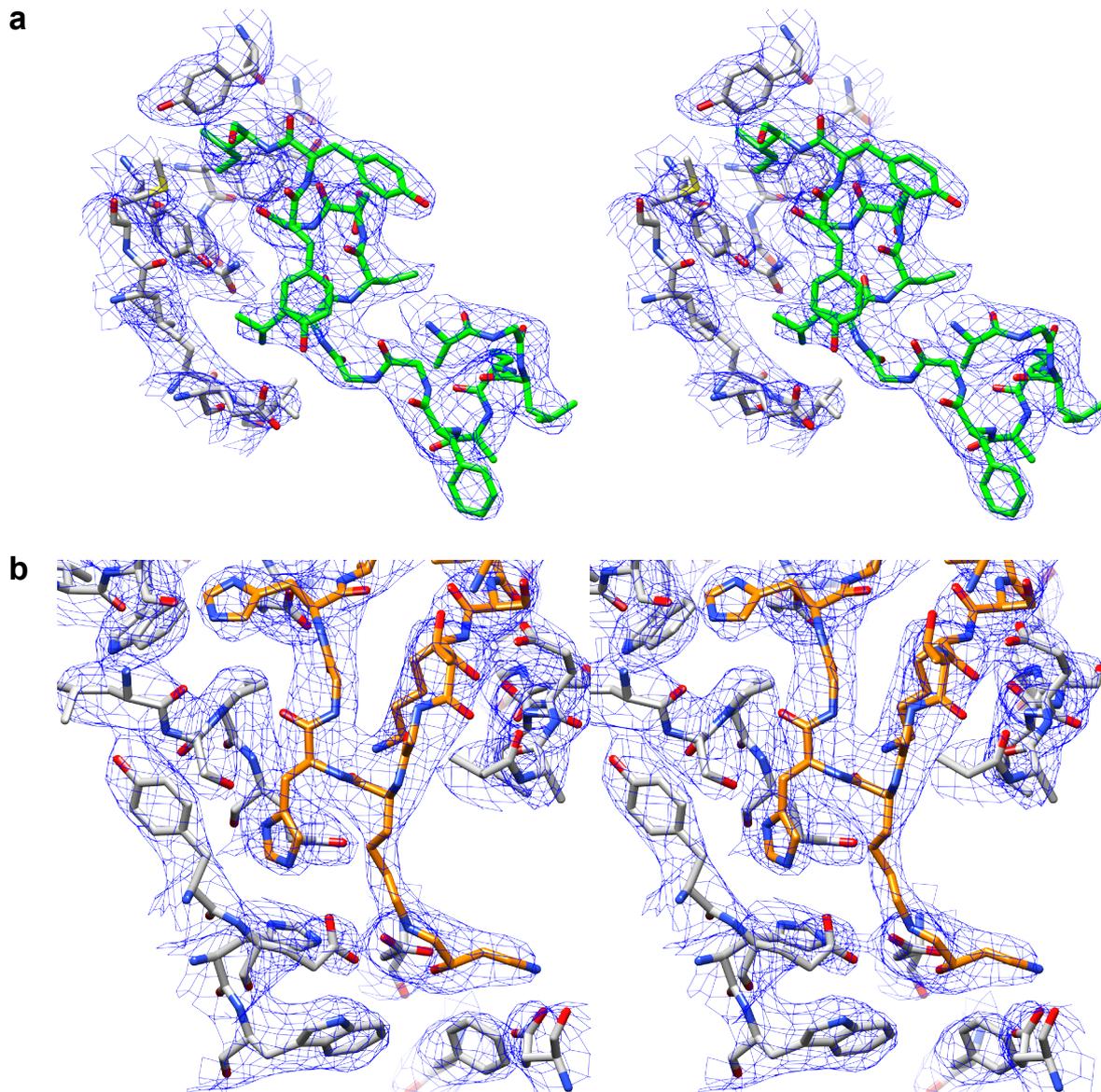
Mouse QODPVIYQRLADAFNKLTASST--PPALDRKQKMAFLKSLSEEFMANV-----GGLLCVK-----1151
 Zebrafish -QODALIYQRLADAFNNLTASST--PPTMDRKQKMAFLKSLSEEFVANV-----GGLLCVK-----1150
 King cobra -QODPIIYQRLADAFNKLTASST--PPTLDRKQKMAFLKSLSEDFMSNVEMNDADDVQPLSSEALAAALQEPYVEQQOQREAKTTPDKTHSHSVGSIEENWQLSQIACVSAP--(132) 1198
 Maize QONPTVKSRLATAFHNLTSNNLSSSLDRPNRQRKNNLSFMADV-----EMNDADDVQPLSSEALAAALQEPYVEQQOQREAKTTPDKTHSHSVGSIEENWQLSQIACVSAP--(132) 1165
 D. discoideum Q--DPSIQSRVVQDEFETLTIIGT-----DRKSKDLMELNQLNFLVNV-----KSLINK-----1133

HEAT 20B HEAT 20C

Chymotrypsin Trypsin

Supplementary Figure 1 Sequence alignment of Xpo4 orthologues.

The alignment includes Xpo4 from mouse (*M. musculus*), zebrafish (*D. rerio*), king cobra (*O. hannah*), maize (*Z. mays*), and *D. discoideum*. Absolutely conserved residues are highlighted in dark red boxes, residues conserved in four out of five sequences in light red boxes. Identified protease cleavage sites are marked with arrows and indicated accordingly. The positions of the HEAT repeat helices of mouse Xpo4 are labeled and positioned below the alignment.

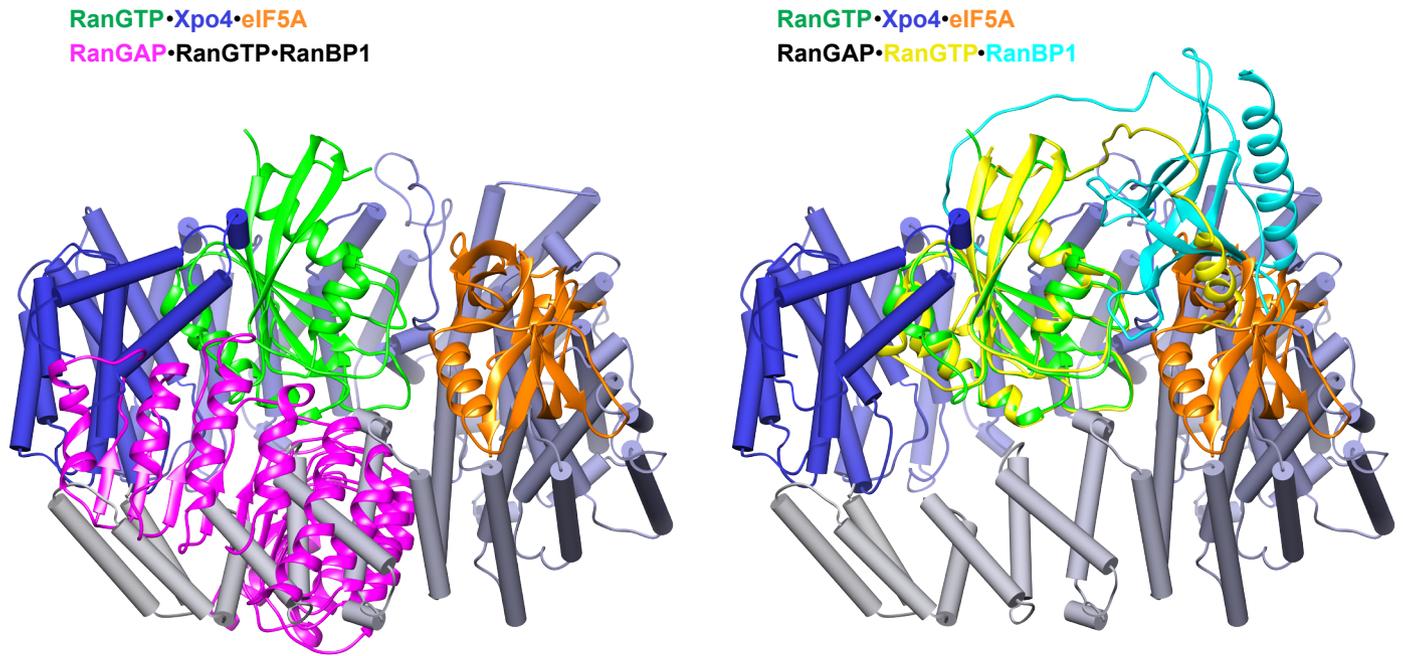


Supplementary Figure 2 Stereo views of the electron density of the export complex structure.

The electron density of the refined 2Fo-Fc map (contoured at 1.0σ) is shown as blue mesh, with the stick representation of the final model superposed.

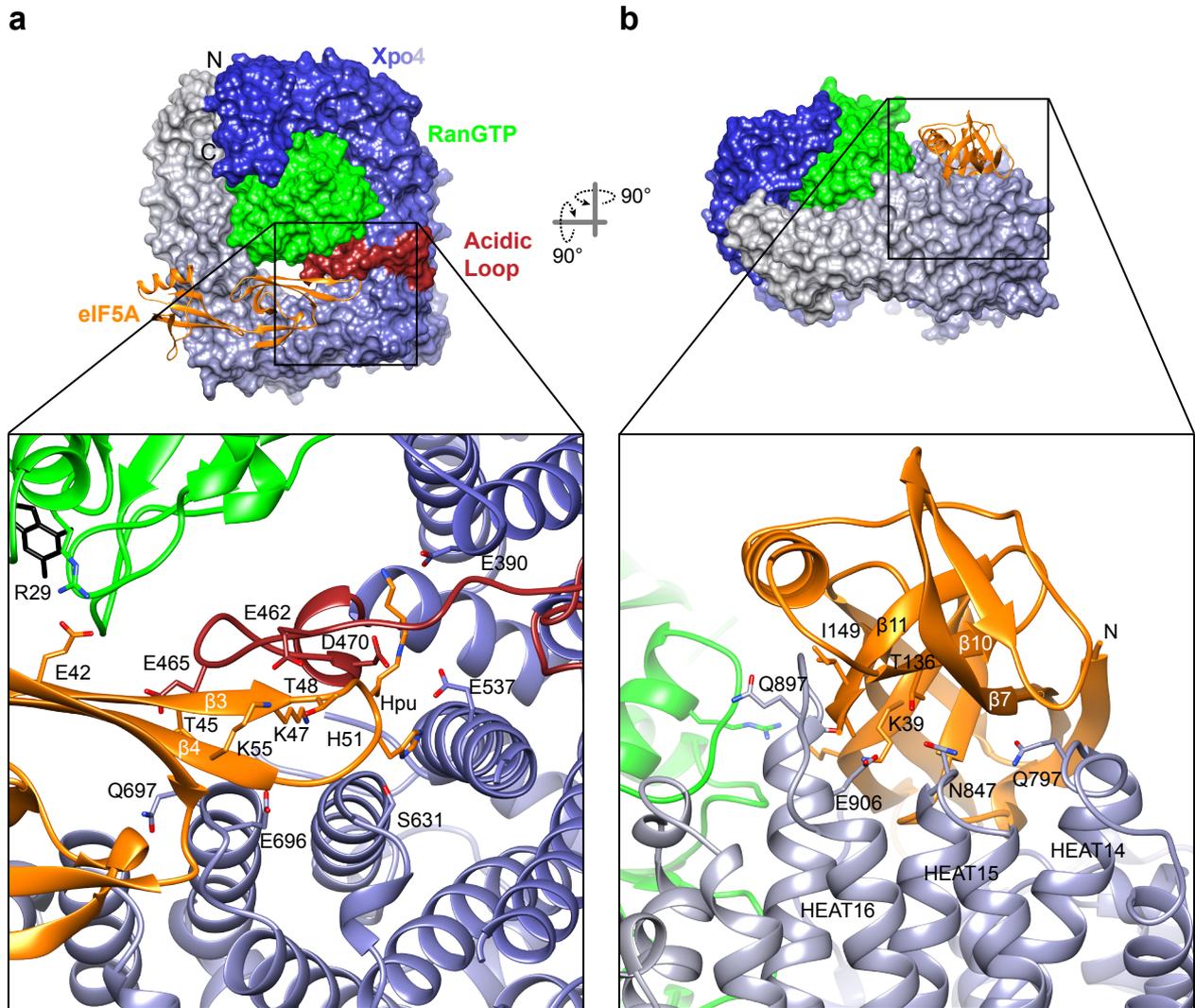
(a) Stereo view of the switch II region of Ran (carbon atoms in green) interacting with Xpo4 (carbon atoms in grey).

(b) Stereo view showing the hypusine-containing loop of eIF5A (carbon atoms in orange) docking into the acidic pocket of Xpo4.



Supplementary Figure 3 Cytoplasmic disassembly of the export complex.

RanGTP•Xpo4•eIF5A complex is overlaid with the RanGAP•RanGTP•RanBP1 structure (PDB ID 1K5D; ref. 1). The structures are aligned via Ran. The eIF5A export complex is shown in a similar color-coding and orientation as in Figure 3 (middle panel). On the left, RanGAP (magenta) is shown in a ribbon representation. On the right, RanGTP is shown in yellow and RanBP1 in cyan. Note the severe clashes of RanGAP with the C-terminus of Xpo4 and of RanGTP•RanBP1 with Xpo4 and eIF5A.



Supplementary Figure 4 Detailed interactions of eIF5A with RanGTP•Xpo4.

Docking of the N-terminal (**a**) and C-terminal (**b**) domains of eIF5A on RanGTP•Xpo4. Xpo4 and Ran are depicted as surface, whereas eIF5A is shown in a ribbon (upper panels). The magnified views (bottom panels) show the interacting residues.

Fig. 5a

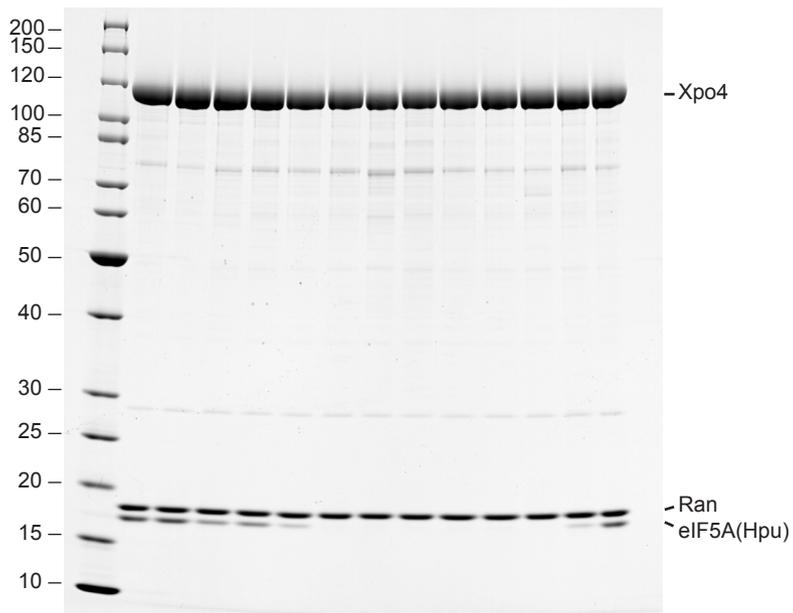
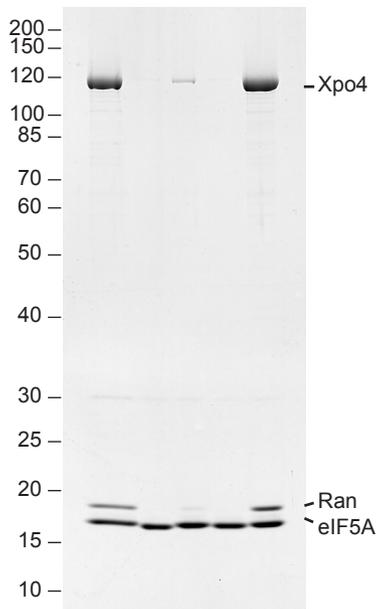


Fig. 5c



Supplementary Figure 5 The original scans of the gels used in Figure 7

Supplementary Reference

1. Seewald, M. J., Korner, C., Wittinghofer, A. & Vetter, I. R. RanGAP mediates GTP hydrolysis without an arginine finger. *Nature* **415**, 662-666 (2002).